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SUGAR TRANSPORT & SUGAR SENSING IN GRAPE

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1. INTRODUCTION

The ripening of grape berries is accompanied by a massive accumulation of soluble sugars, and by the synthesis and accumulation of a wide range of phenolic compounds and aroma precursors. These processes play major roles in the quality of the berries and wine. Sugars are accumulated in the vacuoles of flesh (mesocarp) cells, which account for 65 to 91 % of the fresh weight in a mature berry. Polyphenols accumulate in the skin and seeds, which represent respectively 6 to 20 %, and 2 to 6 % of the berry fresh weight, depending on the cultivars (Galet 1983). Although sugars and polyphenols do not accumulate in the same cells, evidence is growing for some control of polyphenol metabolism by sugars. The expression and activity of sugar transporters mediating sugar accumulation in the berries are also partially controlled by sugars.

During the past few years, molecular approaches have allowed a better understanding of these processes. Furthermore, new sequences encoding so far unidentified factors of these events are raising from the recent release of the complete grape genome (Genoscope data base http://www.cnrs.fr/vitis, Jaillon et al. 2007, Iasma Genomics http://genomics.research.iasma.it, Velasco et al. 2007). These databases, and associated transcriptomic tools, also open the possibility to compare the ripening of a non-climacteric fruit such as grape berry with that of a climacteric fruit such as tomato (*Lycopersicon esculentum*).

After summarizing the physiological basis of sugar accumulation in the

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grape berry, this chapter will give an overview of our present knowledge on the molecular biology of sugar transporters and sugar sensing.

2. PHYSIOLOGICAL BASIS OF SUGAR ACCUMULATION

The ripening grape berry is a strong sink for dry matter transported from current photosynthesis and wood reserves (Coombe 1989). Sucrose derived from leaf photosynthesis is exported *via* the phloem to the berries. From véraison and throughout ripening the berries accumulate roughly equal amounts of glucose and fructose, reaching over 1M of each hexose (Coombe 1987). This implies that phloem transported sucrose is hydrolyzed at some step during its transport from the sieve tube to the vacuole of the mesocarp cell (Fig. 1). The remarkable sink strength of the berry is well illustrated by the fact that its dry mass increases four-fold during a 6 week-period, with little change observed in the dry mass of other plant parts (Conradie 1980).

In situ measurements carried out at different times on the same berry showed that glucose and fructose accumulation begins suddenly on the same day as berry softening begins. It seems that, once begun, sugar accumulation in grapes is undeviating and massive. The limitation to sink strength within individual berries is set by sink activity, not berry size. Indeed, after it is triggered, the accumulation of hexoses is linear with time, although the increase in berry volume proceeds at a variable rate (Coombe 1989).

The fleshy portion of the berry originates from the ovary wall that develops to form the pericarp, mesocarp and endocarp. The developing berry is fed with assimilates transported through the carpellary vascular bundles which are divided into the peripheral and central bundles. A dorsal bundle network extends at the periphery of the fruit, and central vascular bundles are connected to the seeds and irrigate the central flesh. A detailed analysis of the unloading pathway in the ripening berry was conducted in a hybrid grape (*Vitis vinifera* x *Vitis labrusca*; Zhang et al. 2006). A variety of techniques, including electron microscopy, determination of enzymatic activities, and transport studies with carboxy fluorescein (a symplastic tracer) and with companion cell expressed and tagged viral movement proteins, were used. Until véraison, both carboxyfluorescein and the tagged viral movement protein could be released from the functional phloem strands, whereas at the late stage of ripening they remained confined in the strands.

This shift from a symplasmic to an apoplasmic unloading pathway, which occurs at or just prior the onset of ripening, is accompanied by a concomitant increase of the expression and activity of cell wall invertases (cwINV) (Zhang et al. 2006). As a result, apoplastic sugar concentration and osmotic pressure in-

crease, which may enhance sugar uptake *via* stimulation of the proton-pumping ATPase activity (Li and Delrot 1987). Interestingly, a loss of symplastic connections also occurs at the inception of tomato fruit ripening (Ruan and Patrick 1995), with cwINV activity and sugar uptake increasing throughout ripening (Baxter et al. 2005).

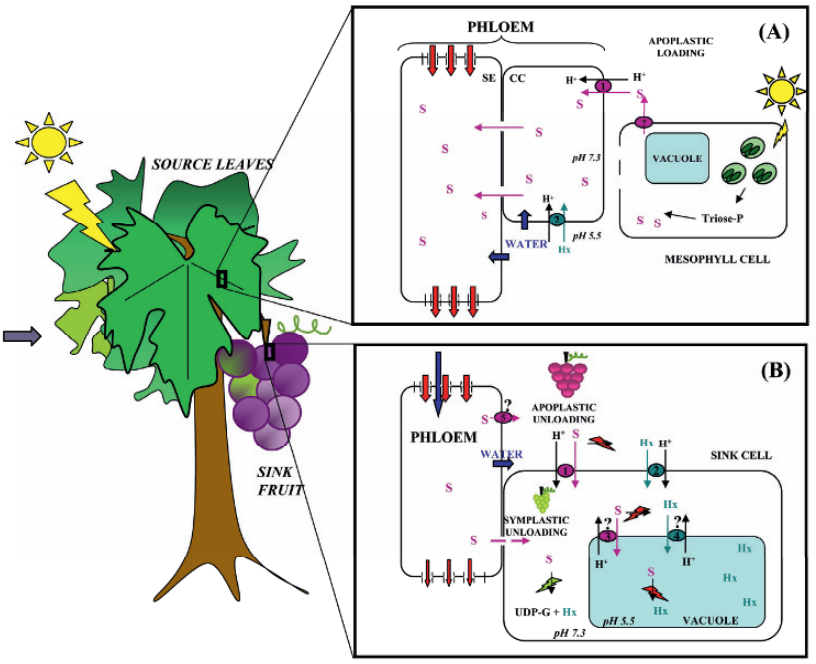

Fig. 1. Simplified scheme of long-distance sugar transport in plants. (A) Pathways for phloem loading: Sucrose (S) is synthesized in mesophyll cells through photosynthesis. S is loaded into the sieve elements/companion cell complex (SE/CC) *via* the apoplast. Apoplastic loading involves the retrieval of S leaking from the mesophyll or the vascular parenchyma (mechanism yet uncertain) and may occur along the phloem path. Hydrostatic pressure drives phloem sap movement toward sink tissue. (B) Pathways of phloem unloading: S enters the receiving cell by the symplastic route before véraison, using plasmodesmata, or the apoplastic pathway after véraison. The latter predominates in the ripening fruit and requires the activity of membrane transporters mediating the transport of S, and of the hexoses (Hx) resulting from S hydrolysis by metabolic enzymes (invertases, sucrose synthases). Hx are accumulated in the vacuole. Water fluxes respond to sugar concentration gradient. (1) S/H^+ symporter; (2) Hx/H^+ symporter; (3) S/H^+ antiporter; (4) Hx/H^+ antiporter; (5) S efflux transporter; invertase; sucrose synthase; water flux.

In grape berries, the analysis of different zones of the skin and flesh showed that the sugar concentrations are heterogeneous in both tissues (Coombe 1987). Concentrations in skin are generally lower than those present in the flesh tissue. Furthermore, there are longitudinal differences in the levels

of glucose and fructose in the outer and central flesh tissues. Hexose concentrations in the flesh increase from low near the brush to high at the stylar end. Sucrose concentration in the flesh is low throughout ripening, but increases in the skin when the berries reach 17 -26° Brix and in the central tissues (including the brush) in overripe berries. Uptake and efflux studies with isolated skin suggested active uptake of D-glucose but not L-glucose. Surprisingly, active uptake of monosaccharides was measured in skin samples from both green unripe and ripe berries, whereas it was evident only in flesh tissues sampled from ripening berries (Coombe and Matile 1980). However, contrarily to what is observed in mesocarp tissues, a high proportion of the sugar absorbed by skin pieces is diffusive, whatever the sampling time.

The limiting steps and the molecular mechanisms leading to final trapping of high concentrations of hexoses in the vacuole of mesocarp cells are still elusive. The compartmentation and enzymatic events leading from sucrose in the sieve tubes to hexoses in mesocarp cell vacuoles involve (a) sucrose efflux from the phloem conducting complex, (b) sugar entry into the flesh cells, and (c) sugar uptake into the vacuoles of these cells. Sucrose may be cleaved in the cell wall prior to its entry into the mesocarp cells, or after compartmentation into the vacuoles. The possibility of sucrose breakdown/resynthesis and final breakdown, often referred to as sucrose futile cycle, should also be considered.

Together with sugar transporters, the sucrose metabolic enzymes invertases (INVs) and sucrose synthases (SuSys) participate in the maintenance of the sucrose gradient needed to sustain mass flow of the phloem sap. Furthermore, as they modulate the pool of available sugars, these enzymes may certainly play important roles in the context of sugar signalling (Delrot 1994, Roitsch et al. 1995, Sherson et al. 2003, Koch 2004). INVs are hydrolases cleaving sucrose into glucose and fructose. In tomato and *Arabidopsis*, INVs are encoded by small gene families with various expression patterns regarding the organ and the environmental and/or metabolic stimuli (Godt and Roitsch 1997, Sherson et al. 2003). These enzymes are either acidic or neutral.

Acidic INVs, such as cwINV and vINV, are localized in the cell wall and in the vacuole, respectively, while the nINV, a neutral form, is present in the cytoplasm. In grape, the cDNA sequence of a cwINV (AY538262) and the promoter region of the gene (EF122148), the complete cDNA sequence of a nINV (*NIN1*, EU016365), as well as 3 incomplete genomic sequences, and 2 vINVs cDNAs (*VvGIN1* AAB47171.1 and *VvGIN2* AAB47172.1) have been cloned (Davies and Robinson 1996, Hayes et al. 2007). Based on protein motif analysis (pfam, Interpro), 10 to 12 encoding putative nINVs and 10 encoding putative acidic INVs genes were found in the grape genome sequence (http://www.genoscope.cns.fr/spip/Vitis-vinifera-whole-genome.html). Although INV activities were considered to be non-limiting (Davies and Robinson, 1996), re-

cent reports by Zhang et al. (2006) and Hayes et al. (2007) clearly indicate that expression and activity of cwINV is induced just prior to véraison.

However, although the co-regulation of cwINV and some monosaccharide transporters in sink tissues has been confirmed in several plant species and organs, including young grape leaves, the same could not be demonstrated in grape berries (Fotopoulos et al. 2003, Weschke et al. 2003, Roitsch and Gonzalez 2004, Baxter et al. 2005, Hayes et al. 2007). Furthermore, cwINV enzyme activity in berry represents only 4% of total INV activity (Rüffner et al. 1990, Davies and Robinson, 1996) and recent microarray results showed a constant level of both *cwINV* and *nINV* mRNAs throughout berry development (Deluc et al. 2007). This suggests that cwINV alone cannot be responsible for the increase of monosaccharide concentration in the ripening berry. Acidic vINV activity, although participating in sink activity and strength, as the major sucrolytic activity in grape berry, occurs too early to trigger by itself hexose accumulation at ripening inception (Rüffner et al. 1990, Davies and Robinson 1996, Patrick 1997, Dreier et al. 1998). *VvGIN1* and *VvGIN2* transcripts and protein levels accumulate before véraison and decrease during fruit ripening (Davies and Robinson, 1996, Sarry et al. 2004, Deluc et al. 2007). However, vINV activity is important to drive the import of sugars along ripening, since its natural reduction in the Steuben grapevine hybrid contributes to an increase of the relative proportion of sucrose in the maturing berry (Takayanagi and Yokotsuka 1997). Likewise, vINV activity in tomato fruit is crucial to determine both the levels and the nature of accumulated sugars in the vacuole (Klann et al. 1993, Ohyama et al. 1995, Nguyen-Quoc and Foyer 2001).

SuSys are cytosolic glycosyl transferases which, in the presence of UDP, convert sucrose into UDP-glucose (UDP-G) and fructose. *In vivo*, SuSys are also able to catalyse the reverse reaction, but with lower efficiency (Geigenberger and Stitt 1993). Unlike INVs, SuSy enzymes are active and transcriptionally upregulated under low oxygen conditions. Interestingly, *SuSys* that respond to low oxygen are also highly expressed under carbohydrate depletion (Zeng et al. 1999, Koch et al. 2000). In addition, SuSy activity is known to be involved in key metabolic processes such as storage, defence and cell wall synthesis since its UDP-G product is implicated in the formation of callose and in the synthesis of several cell-wall polysaccharides (Albrecht and Mustroph 2003).

In tomato, SuSy but not INV, is involved in sugar unloading and metabolism at the beginning of fruit development (Dali et al. 1992, D'Aoust et al. 1999, N'tchobo et al. 1999). Later in maturation, SuSy activity is strongly reduced but sucrose unloading rates, although low, are maintained, and might be driven by both sugar uptake and INV activities (D'Aoust et al. 1999, Nguyen-Quoc and Foyer 2001). In grape, 6 to 9 genes encoding putative SuSys are expected based on recent genome sequencing. However, SuSys activities do not vary much along ripening and are not conclusively correlated with the modifica-

tions on the soluble sugar concentration (Zhang et al. 2006). For instance, the expression of a *SuSy* gene homologue to *CiTSUA* (1609402_at, TC62599, http://www.plexdb.org/modules/PD_browse/experiment_browser.php? plex_name=GrapePLEX) increases gradually along berry development. However, whether the subsequent enzyme is involved in sugar import or cell wall expansion during berry softening is not clear. Hence, it appears that sugar accumulation in berry, and sink organs in general, would rather result from the coordinate action of several mechanisms, involving various transporters and hydrolytic enzymes (Deluc et al. 2007).

The simultaneous events of sucrose (or starch) synthesis and degradation occurring in plants cells are sometimes referred to as 'futile cycle'. Although SuSy is able to catalyze the synthesis of sucrose, in the cytosol this sugar is mainly synthesized by sucrose phosphate synthase (SPS) (Huber and Huber 1996). This holds true both for photosynthetic tissues and for non-photosynthetic storage organs. Sucrose futile cycle implicates the re-synthesis of sucrose from the hexoses present in the cytosol, its entry in the vacuole and its degradation by vINV. A continuous sugar exchange between the cytosol and the vacuole (sucrose influx, hexose efflux) has also been suggested in tomato pericarp cells (Scholes et al. 1996). Sucrose futile cycles would then favour the unloading and storage of sugars into the ripening fruit which becomes symplastically isolated (Nguyen-Quoc and Foyer 2001). In grape berry, an *SPS* gene is preferentially expressed in the pericarp, suggesting a probable participation in the re-synthesis of sucrose following its unloading from the phloem into the fruit (Grimplet et al. 2007).

3. MOLECULAR BIOLOGY OF SUGAR TRANSPORTERS

3.1. Monosaccharide transporters (MSTs)

In higher plants, *AtSTP1* (*Arabidopsis thaliana* Sugar Transporter Protein 1) was the first MST cDNA identified and functionally characterized as a hexose-proton symporter, by complementation of hexose transport null-mutant yeasts (Sauer et al. 1990). Since then, many clones have been identified in various plant species where they belong to multigene families: the *Arabidopsis* genome display 53 homologous sequences encoding putative MSTs, distributed into 7 distinct clusters (Büttner 2007).

They all share a structure of 12 trans-membrane domains with N- and C-cytoplasmic termini characteristic of all sugar transporters belonging to the MFS (Major Facilitators Superfamily, Williams et al. 2000, Delrot et al. 2001). In grape, 59 putative hexose transporters encoding genes have been identified based on protein motif recognition (Samson et al. 2004, Jaillon et al. 2007). Six

full length cDNAs encoding for MST and named *VvHT1* to *6* (*V. vinifera* Hexose Transporter, *VvHT1* AJ001061, *VvHT2* AY663846, *VvHT3* AY538259 and AY854146, *VvHT4* AY538260, *VvHT5* AY538261, *VvHT6* AY861386, DQ017393) were previously cloned from various grape cultivars such as Pinot noir, Ugni blanc, Chardonnay, Cabernet Sauvignon and Syrah (Fillion et al. 1999, Vignault et al. 2005, Hayes et al. 2007).

The predicted peptides share about 60% homology to each other (Büttner and Sauer 2000, Büttner 2007). Interestingly, computer analysis revealed the presence of N-terminal signal peptide for VvHT3 and VvHT4 and a 200 aminoacid cytoplasmic extension in the centre of the VvHT6 protein, between transmembrane helices 6 and 7 (Hayes et al. 2007). Except the latter one, all VvHTs identified so far present high homologies with AtSTPs, *i.e.* functional hexose transporters (Fig. 2). VvHT6 is related to AtTMT2, a member of the TMT (Tonoplast Monosaccharide Transporter) subfamily of MFS transporters. AtTMTs are tonoplastic hexose-proton antiporters and possess a long hydrophilic central loop. These transporters are induced by abiotic stresses such as cold or drought and were suggested to play a role as sensors (Wormit et al. 2006).

A cDNA encoding a putative plastidic glucose transporter (*pGLT* AY608701), sharing high homology with *AtpGLcT* (CAC01856) has also been reported (Terrier et al. 2005, Glissant 2005). Another *pGLT*-like transcript (1608991_at, TC60060) is highly expressed during berry maturation (Deluc et al. 2007). Consistent with the observed increase of starch metabolism during berry development, *Vv*pGLT-like genes would encode plastidic transporters involved in the export of glucose resulting from starch degradation (Weber et al. 2000, Deluc et al. 2007). Eventually, Deluc and co-workers (2007) identified a transcript homologous to a sugar/polyol membrane transporter (*At*PLT5), whose expression increases along berry development (1613408_at, TC66667, http://www.plexdb.org/moules/PD_browse/experiment_browser.php?plex_name=GrapePLEX, Deluc et al. 2007).

Uptake activities of VvHT1, VvHT4 and VvHT5 have been demonstrated by heterologous expression in the *hxt-null* mutant yeast EBY VW 4000 (Wieczorke et al. 1999). All three VvHTs are high affinity, H^+-dependent transporters mediating the uptake of radiolabelled D-[U-^{14}C]glucose according to saturable Michaelis-Menten kinetics. VvHT1 exhibits the highest affinity for glucose (K_m of 70 µM, V_{max} about 14 µmol min^{-1}g FW^{-1}) compared to VvHT4 and VvHT5 (K_m about 150 µM and 100 µM respectively, V_{max} about 5 and 0.15 µmol min^{-1} g FW^{-1}, respectively) and is the only one able to restore the growth of the complemented yeast on glucose. VvHT3 was not able to transport any of the tested radiolabelled sugars in the deficient yeast model (Vignault et al. 2005, Hayes et al. 2007). Up to date, attempts to confirm the transport activity of both VvHT2 and VvHT6 in yeast had little success.

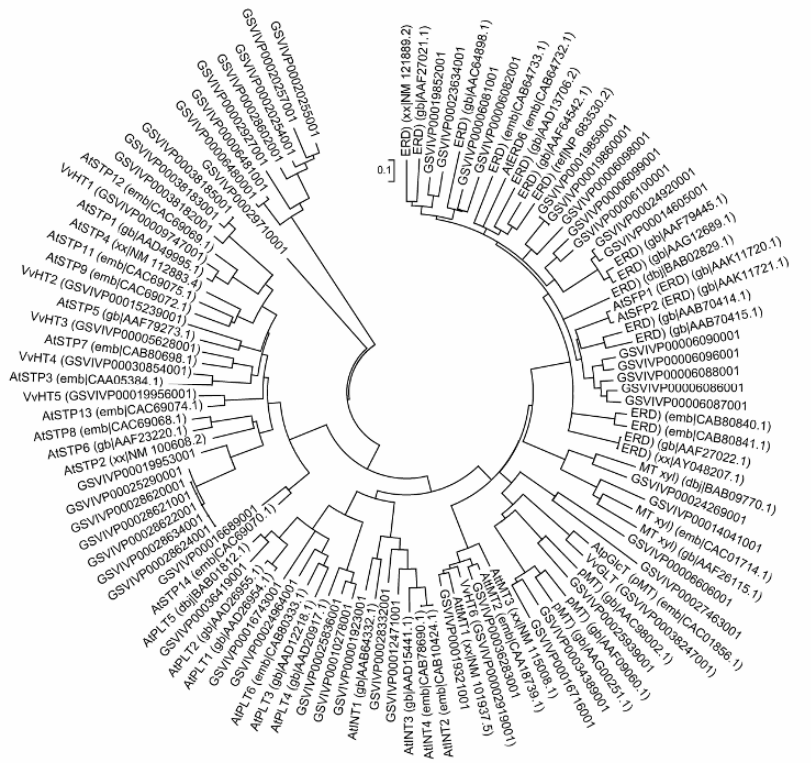

Fig. 2. MST evolutionary relationships of 111 taxa from *A. thaliana* and *V. Vinifera*.The evolutionary history was inferred using the Minimum Evolution method (Rzhetsky and Nei 1992). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling 1965) and are in the units of the number of amino acid substitutions per site. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm (Nei and Kumar 2000) at a search level of 1. The Neighbor-joining algorithm (Saitou and Nei 1987) was used to generate the initial tree. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 430 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007) from initial multiple alignments realised with muscle (Edgar 2004). Disclaimer: Although utmost care has been taken to ensure the correctness of the caption, the caption text is provided "as is" without any warranty of any kind. Authors advise the user to carefully check the caption prior to its use for any purpose and report any errors or problems to the authors immediately (www.megasoftware.net). In no event shall the authors and their employers be liable for any damages, including but not limited to special, consequential, or other damages. Authors specifically disclaim all other warranties expressed or implied, including but not limited to the determination of suitability of this caption text for a specific purpose, use, or application.

VvHT1 displays broad substrate specificity, being able to transport galactose, xylose and glucose analogs such as 3-*O*-methyl glucose. Mannose competes with glucose for the active binding site but is not transported through the membrane. Fructose does not significantly affect glucose uptake rates in the mutant yeast expressing *VvHT1*, whereas it behaves as a competitive inhibitor in grape cell suspensions (Vignault et al. 2005, Conde et al. 2006, Hayes et al. 2007). Furthermore, uptake of radiolabelled D-[U-^{14}C]fructose in grape cells also display Michaelis-Menten kinetics with a V_{max} similar to the one measured for glucose uptake, suggesting that both monosaccharides are transported by the same protein, although the K_m for fructose was much higher (Conde et al. 2006). However, AtSTP1, its closest relative in *A. thaliana*, is unable to transport fructose (Boorer et al. 1994). The involvement of an additional monosaccharide transporter sharing similar characteristics in the grape suspension cells model might explain these results (Conde et al. 2006). VvHT4 transport activity may be restricted to glucose, which constitutes a major difference from its *Arabidopsis* homologue, AtSTP3 displaying low affinity for glucose (Büttner et al. 2000). Conversely, both glucose and fructose are substrates of VvHT5 (Hayes et al. 2007).

The plasma membrane localization expected for these three VvHTs, as well as for the non-functional VvHT3, has been confirmed by immunofluorescence, immunolabelling and C-terminal GFP fusion (Vignault et al. 2005, Hayes et al. 2007). VvHT2 and VvHT6 appear to be expressed in the tonoplast (Vignault et al. unpublished) whereas VvpGLT resembles typical chloroplastic translocators. The involvement of tonoplast sugar transporters is expected because the vacuole is the major compartment of hexose storage in berry flesh during ripening. Furthermore, the recent characterization of the tonoplastic glucose-proton antiporter AtVGT1 (At3g03090) in *A. thaliana* supports the involvement of such mechanisms in other species (Aluri and Büttner, 2007).

The expression of hexose transporters is mainly associated with sink tissues where they import the unloaded sugars from the apoplast. In grape berry, *VvHT1*, *VvHT2* and particularly *VvHT3* are highly expressed compared to the other *VvHT*s, at all developmental stages. *VvHT2* mRNA levels remain constant throughout berry development. However, recent data report that *VvHT2* expression is most specifically associated with véraison (Terrier et al. 2005, Hayes et al. 2007, Vignault et al. unpublished).

Nonetheless, some specific expression patterns can be distinguished: both *VvHT1* transcripts and protein levels (Conde et al. 2006) are much higher at pre-véraison stages, whereas *VvHT5* mRNA accumulation, although weak, is mostly associated with late ripening days. *VvHT3* mRNA levels are sharply reduced at véraison but high at both green and ripening stages (Hayes et al. 2007), whereas *VvHT6* transcripts are highly accumulated at véraison, suggesting that this transporter may be responsible for early import of hexose at the inception of

ripening (Vignault et al. 2005, Terrier et al. 2005, Deluc et al. 2007). *VvHT1* is expressed in the plasma membrane of flesh cells mainly at green stages and provides sugar to cells when energy is required to complete cell division and growth. However, this transporter is barely detectable in plasma membrane fraction of ripening berries and thus cannot be directly responsible for the post-véraison sugar accumulation (Vignault et al. 2005, Conde et al. 2006).

Monosaccharide import into berries during ripening would be mainly due to the VvHT2 and VvHT3 transporters although their sugar transport activity has not yet been experimentally demonstrated. To a lesser extent, *VvHT4*, although weakly expressed, and *VvHT5* could also be involved (Hayes et al. 2007). In spite of the high number of putative MST genes in the grape genome, no other VvHT was identified in grape berries, suggesting that the most important transporters for sugar accumulation in this organ have already been cloned. In addition to their expression profiles, which give some clues about their physiological role, their tissue and cell specific expression needs to be determined. The diversity of hexose transporter genes expressed along berry development is consistent with the shift from a symplastic to an apoplastic phloem unloading pathway that occurs prior to véraison, the later being the dominant mechanism of sugar import into cells at ripening stages (Bondada et al. 2005, Zhang et al. 2006).

In both young and mature leaves, *VvHT1* and *VvHT3* are the most abundant *VvHTs* transcripts. Expression of *VvHT1*, *VvHT3* and *VvHT5* is enhanced with the transition from sink to source activity. Conversely, *VvHT2* transcript levels, constantly weak, seem to be preferentially associated with sink activity (Hayes et al. 2007). *In situ* hybridization showed that *VvHT1* transcripts are abundant in the phloem region of the conducting bundles of the leaf, petiole and berry (Vignault et al. 2005). Although the phloem sap mainly contains sucrose, minor amounts of monosaccharides are also detected, and VvHT1 could participate in the retrieval of hexose leaking from the conducting complex. Its high affinity and its particular location support that hypothesis.

3.2. Sucrose transporters (DSTs)

In plants, SoSUT1 (*Spinacia oleracea* Sucrose Transporter 1) was the first disaccharide transporter (DST) functionally characterized in a yeast mutant deleted for invertase and expressing sucrose synthase (Riesmeier et al. 1992). DSTs genes, which belong to small multigenic families with 9 members in *Arabidopsis* and 4 in tomato for instance, encode for a 55 kD polypeptide (*Arabidopsis* Genome Initiative 2000, Delrot et al. 2001, Sauer et al. 2004, Hackel et al. 2006). Plant DSTs can be clustered into 4 groups regarding their protein sequence homologies: group 1 and group 2 are exclusively composed by

monocots and dicots transporter proteins, respectively, whereas groups 3 and 4 are mixed. Group 3 clusters proteins possess longer N-termini and central cytoplasmic loops whereas group 4 sequences display shorter C-termini (Barth et al. 2003).

Group 3 transporters have been reported to localize all along the sieve element and have been proposed to sense the sucrose flux through the plasma membrane (Schulze et al. 2000). Group 4 sucrose transporters are low affinity high capacity transporters (LAHC) localized in the membranes of minor veins of source leaves (Weise et al. 2000). However, their subcellular localization is controversial since, although correctly directed to the yeast plasma membrane, some of these proteins may be specific to the tonoplast in plants (Endler et al. 2006). Up to date, three *DST* cDNAs have been cloned from Shiraz and Cabernet Sauvignon (*VvSUC11*; AF021808, also identified as *VvSUT1* AF182445; *VvSUC12* AF021809; *VvSUC27*, AF021810) and characterised as proton-dependent sucrose transporters, whereas 9 DSTs sequences are present in *Arabidopsis* genome (Sauer et al. 2004). VvSUC11 and VvSUC12 are intermediate affinity sucrose transporters (K_m, 0.9 mM and 1.4 mM, respectively) (Ageorges et al. 2000, Manning et al. 2001), whereas VvSUC27 has a K_m of about 10 mM and is thus a low affinity sucrose transporter (Zhang et al. 2008). The grape genome sequence recently released (Jaillon et al. 2007, Velasco et al. 2007) suggests that sucrose transporter genes would constitute a small multigenic family of 4 members in this species (Fig. 3).

VvSUC11 is expressed in flowers and fruits whereas *VvSUC12* expression is restricted to berries and young leaves. In addition, *VvSUC11* is expressed in both young and expanded leaves. *VvSUC27* expression is closely related to sink activity since its transcripts are strongly accumulated in flowers and unripe berries, roots and tendrils but poorly present in mature leaves (Davies et al. 1999). *VvSUC27* expression is associated with the early stages of berry development, *VvSUC11* and *VvSUC12* transcription concomitantly increases with post-véraison sugar accumulation, which suggests a direct pathway for sucrose acquisition by berry cells (Davies et al. 1999). However, information regarding sucrose uptake in berry along ripening is scarce. Sucrose uptake activity has been demonstrated in berry slices (Conde et al. unpublished results) but further investigation, such as sucrose transporters localization in berry flesh is needed (Hayes et al. 2007).

Indeed, even if most of the sucrose transporters yet characterised are responsible for the loading of phloem conducting cells, the involvement of sucrose carriers into the uptake by the storage cells of sugars unloaded at the end of the phloem path has been described [*e.g.* DcSUT2 in carrot (Shakya and Sturm, 1998), StSUT1 in potato (Kühn et al. 1997), CiSTU2 in Citrus (Li et al. 2003), AtSUC3 in *A. thaliana* (Meyer et al. 2004) and LeSUT1 in tomato (Hackel et al. 2006)]. The presence of SUT transporters in sucrose releasing

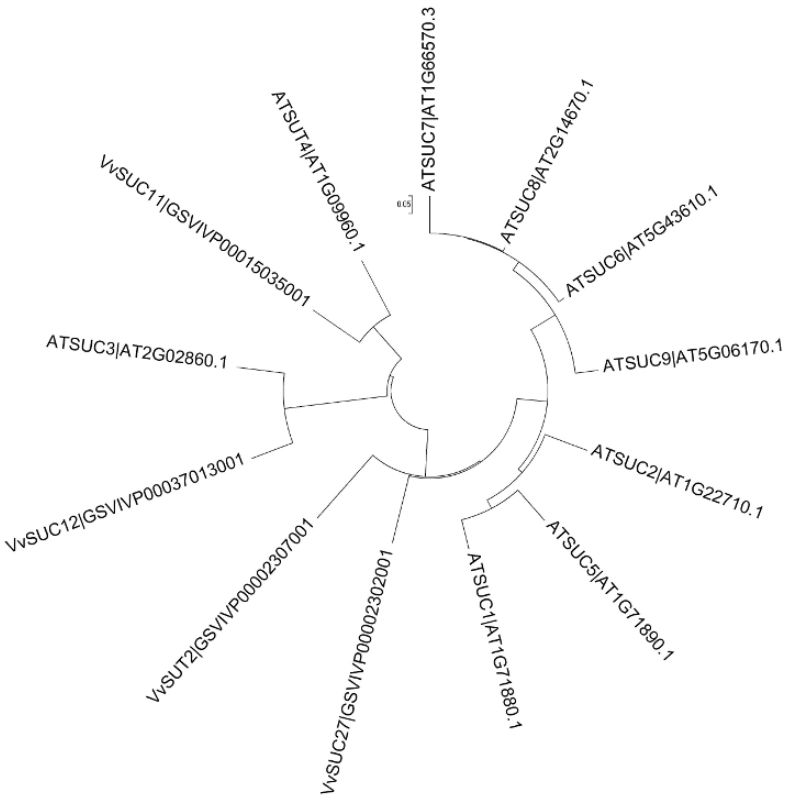

Fig. 3. DST evolutionary relationships of 13 taxa from *A. thaliana* and *V. vinifera.* For a legend, see Fig. 2.

tissues of cereals (Bagnall et al. 2000, Aoki et al. 2006) and the demonstration that AtSUC2 loss-of-function and StSUT1 sink-specific antisense repression affect phloem unloading in *Arabidopsis* and potato, respectively (Gottwald et al. 2000, Kühn et al. 2003) suggest that some of the SUT proteins can be directly involved in phloem unloading and thus may function as reversible transporters. Indeed, Carpaneto and co-workers expressed *ZmSUT1* (orthologue of AtSUC2) in *Xenopus* oocytes and succeeded in inversing its transport mode (Carpaneto et al. 2005).

The involvement of a classical sucrose/proton symporter in the proton-independent sucrose efflux activity observed in potato plasma membrane vesicles has also been suggested. This protein would then act in a different way under certain conditions, as it has been reported for the CkHUP1 glucose transporter (Komor and Tanner 1974, Kühn et al. 1997). Since sucrose unloading occurs down the sucrose gradient, sucrose efflux involves biochemical mechanisms that differ from those driving its uptake into sucrose accumulating tissues and the functional asymmetry of these transporters would be driven by specific

membrane potential and gradients (Carpaneto et al. 2005). Furthermore, the roles of a putative sucrose/proton antiporter and of a non-selective channel has also been suggested in seed coats of broad bean and pea, respectively (De Jong et al. 1996, Walker et al. 1995, 2000). Eventually, the participation of DSTs as retrievers of unloaded sucrose into the apoplasm, or as direct modulators of sucrose concentration and sink strength is well demonstrated in sink tissues (Lalonde et al. 2003).

4. SUGAR SENSING AND REGULATION OF TRANSPORTERS

Because sugar producing and sugar consuming and/or storage organs are spatially separated, plants have developed ways to understand their "sugar status" in order to adapt carbohydrate synthesis and transport. In a manner resembling the well-known glycaemia regulation in mammals, plants sense their sugar levels, and modulate them according to the general "feast and famine" pattern of responses: (a) carbohydrate excess favours the expression of enzymes connected with biosynthesis and storage of reserves and (b) represses transcripts encoding enzymes involved in photosynthesis and reserve mobilisation (Koch 1996).

Additionally, sugars provide signals able to control many aspects of seed and plant development. The mechanisms of sugar perception and the subsequent transduction pathways regulated are referred to as sugar sensing and signalling, respectively (Smeekens 2000). These terms, however, qualify for a complex scheme regarding (a) the diversity of perception mechanisms and levels involved, (b) the molecular pathways induced, (c) the multilevel control of gene and protein expressed and, (d) the interactions with pathways mediating the transduction of hormonal and environmental stimuli. The study of the molecular mechanisms of sugar sensing and signalling pathways in plants takes advantage of the well-known pathways described in yeasts (Rolland et al. 2006, Santangelo 2006). The following paragraphs give an overview of the sugar control over sugar transport and polyphenol metabolism in grapevine.

4.1. Sugar regulation of sugar transport

A fine way to control resource distribution among tissues and organs is to regulate their transport throughout the plant (Koch 1996). The mechanisms by which sugars regulate their own transport proteins have been extensively studied in yeast and mammals but much less in plants, even if many examples now provide evidence for a fine and complex action of sugars (*e.g.* BvSUT1,

Vaughn et al. 2002). Furthermore, altered concentrations of the same sugar can dually control the same target (*e.g*. VfSUT1, Weber et al. 1997) and/or differentially affect the expression of the plasma membrane transporters, from gene transcription to correct targeting and/or activity of the protein itself (for review see Rolland et al. 2006).

In grape suspension cultured cells, *VvHT1* expression is tightly controlled by sugars: (a) high glucose concentration (150 mM) represses *VvHT1* transcription through a pathway mediated by the hexokinase (HXK) sensor, a cytosolic enzyme that also independently catalyses the first step of glycolysis, (b) physiological concentrations (58 mM) are essential to maintain basal levels of *VvHT1* transcription (Atanassova et al. 2003), and (c) high glucose concentration decreases glucose uptake activity and this inhibition is at least in part due to the decrease of VvHT1 protein amount in the plasma membrane *via* an HXK-independent pathway (Conde et al. 2006). The dual transcriptional regulation of *VvHT1* by glucose levels is similar to the control of *HXT2* and *KHT2* expression described in the yeasts *Saccharomyces cerevisiae* and *Kluyveromyces lactis*, respectively (Wendell and Bisson 1994, Milkowski et al. 2001).Indeed, the expression of the high affinity glucose transporters HXT6 and HXT7 in *S. cerevisiae* is induced by very low external sugar concentrations (< 50 mg/L) and repressed by higher concentrations, whereas the low affinity transporter HXT1 is transcriptionally upregulated by glucose concentrations up to 100 mg/L. HXT2 and HXT4, are expressed at glucose concentrations up to 100 mg/L but repressed by higher glucose concentrations. HXT3 expression does not depend on glucose stimulation (Boles and Hollenberg 1997, Rolland et al. 2002, Klockow et al. 2008). In grape, sugars also control the expression of the other *VvHTs*, although their responses are usually weaker (Fig. 4). In suspension cells, *VvHT2*, *VvHT3* and *VvHT6* transcripts transiently accumulate under sugar starvation. Furthermore, whereas *VvHT3* is repressed by high glucose concentration, *VvHT6* expression is strongly induced by high glucose levels (Conde et al. 2006). This recalls the strong expression of *VvHT6* in the fruit at the véraison stage, a step characterized by the sudden and sharp import of sugar into the berry (Terrier et al. 2005, Vignault et al. 2005, Hayes et al. 2007). *VvHT4* and *VvHT5* do not respond to any sugar treatment in grape suspension cells, and appear therefore insensitive to sugar regulation in this experimental system (Conde et al. 2006).

Sugar (sucrose or glucose) induction of *VvHT1* transcription may be mediated by a putative plasma membrane sugar sensor. Indeed, palatinose, a non-metabolizable and non-transportable sucrose analogue, upregulates *VvHT1* in the same way as sucrose (Atanassova et al. 2003). Membrane sugar sensors are well known in yeasts, and to a lesser extent in mammals (GLUT-2 and SGLT3, Guillemain et al. 2000, Diez-Sampedro et al. 2003). They have been also identi-

fied in filamentous fungi (Madi et al. 1997). In *S. cerevisiae*, the modified glucose facilitators SNF3 and RGT2 are unable to transport sugar but possess a C-terminal specific extension giving them the ability to sense glucose as a signal (Coons et al. 1997, Özcan et al. 1998, Rolland et al. 2002).

Furthermore, each sensor displays different glucose affinity and thus would initiate adapted responses. SNF3, as a high affinity glucose sensor, is required for the transcription of high affinity transporters such as HXT2 and HXT4, at low glucose concentration and its own transcription is repressed by high glucose concentrations. Conversely, RGT2 is constitutively expressed and integrate high sugar levels as inducers of the expression of low sugar affinity facilitators such as *HXT1*. The importance of both sensors is underlined by the fact that the double mutant *snf3rgt2* is unable to maintain any HXT in its plasma membrane (Özcan et al. 1998, Rolland et al. 2002).

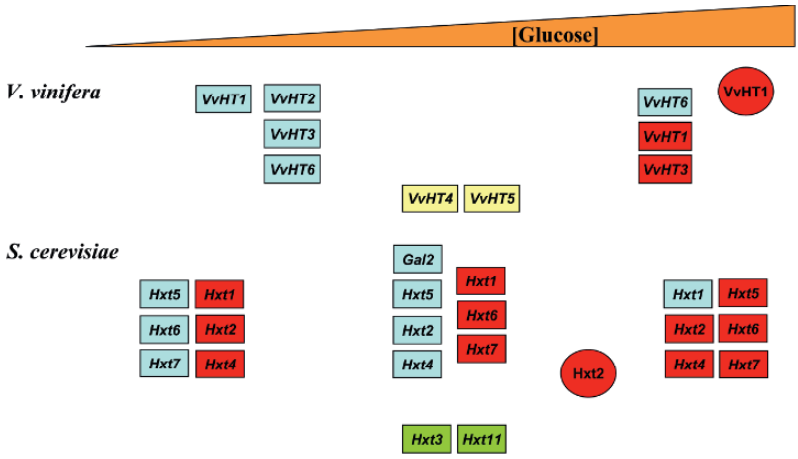

Fig. 4. Expression patterns of glucose transporters in *V. vinifera* and *S. cerevisiae* according to glucose availability. Glucose levels affect both gene (rectangles) expression and protein (circle) amounts. Some transporters are induced (blue) and/or repressed (red) by different levels of glucose whereas others are constitutively expressed (green) or not regulated by sugar concentrations (yellow).

The occurrence of similar sensors, suggested by the effects of poorly permeant sugar analogues, cannot be ruled out in plants. A role as sugar sensors was proposed for SUT2/SUC3-type transporters. Indeed, the tomato protein LeSUT2 was thought to be a sensor because (a) its structure, resembles the yeast SNF3 and RGT2 proteins (*i.e.* unusual protein extensions), (b) its transport inability once expressed in baker's yeasts, and (c) its colocalisation with the functional transporters LeSUT1 and LeSUT4 in sieve elements (Barker et al. 2000). The poor efficiency of AtSUT2 (orthologue of LeSUT2) led to modify the putative role of the SUT2/SUC3-type transporters from orthodox sensors to flux

sensors, measuring the transport rates of sucrose across the membrane (Meyer et al. 2000, Schulze et al. 2000). The capacity of LeSUT2 to interact with other transporters was suggested to be a putative mechanism to modulate sucrose import into the phloem (Reinders et al. 2002). However, none of these arguments are sufficient to confer such a specific role to SUT2/SUC3-type transporters: (a) although no specific function could be attributed to the amino acid extension of these proteins, it did not interfere with the transport function, (b) the poor efficiency of the transport in baker's yeasts depends on the correct expression of the heterogously expressed protein, (c) the colocalization is not a general feature of these transporters, as demonstrated in *Plantago major*, where PmSUC3 and PmSUC2 are expressed in different cell types and their respective genes at different developemental stages (Barth et al. 2003, Eckardt 2003).

Likewise, the role of some AtTMT monosaccharide transporters as putative glucose sensors has been suggested but still remains elusive (Wormit et al. 2006). In yeast and mammals, other proteins than sugar transporter-derived sensors are able to detect sugars as signals and initiate responses. Such membrane proteins are members of the G protein coupled-receptors (GPCR) family (Rolland et al. 2006, Le Gall et al. 2007). In plants, the sensor AtRGS1 mediates HXK-independent glucose signalling and plays a role in *A. thaliana* development (Chen and Jones 2004, Johnston et al. 2007). AtRGS1 acts as a glucose sensor and regulates the GTPase activity of AtGPA1, a GTP-binding protein. Indeed, AtRGS1 is a plasma membrane receptor that interacts with AtGPA1 upon glucose binding to hydrolyse GTP and control of cell proliferation. Interestingly, the AtRGS1 protein is also involved in ABA signalling in seed germination (Chen et al. 2006). A homologue of AtRGS1 was identified in the grape genome (CAO46706.1) by conserved domain homologies.

The hexose content is considered as a regulator of cell division and expansion whereas the sucrose concentration is related to maturation and differentiation processes (Wobus et al. 1999). Controlling the concentrations of these sugars may then be a way to direct tissue differentiation. Sucrose metabolizing enzymes, because they modulate these pools, are natural candidates to be involved in sugar sensing mechanisms. It is therefore not surprising that the genes encoding for these proteins are under sugar control (Roitsch et al. 2003, Koch 2004). For instance, the maize *vINV* gene, *Ivr1* is repressed by sugar but transcriptionally enhanced by sugar depletion. However, its homologue *Ivr*2 is induced by addition of sugar. Similar patterns have been shown for the *SuSy* genes, *Sh1* and *SUS2* (Xu et al. 1996). Interestingly, sugar-mediated regulation of *Ivr2* expression may occur in water stressed leaves (Kim et al. 2000, Trouverie et al. 2004).

The complex response of *VvHT1* to sugar signals correlates with the presence in its promoter of 4 putative *cis*-elements previously found in the promot-

ers regions of sugar-modulated genes (Fillion et al. 1999, Delrot et al. 2000, Atanassova et al. 2003): (a) the positive sugar-responsive *cis*-elements *SURE1* first identified in the promoter of the potato storage proteins patatin (Grierson et al. 1994), and *Sucrose box 3*, identified in the promoter of petunia chalcone synthase (*CHS*) gene (Tsukaya et al. 1991), and (b) the motifs *AMYBOX2* (*TATCCA*) and *AMYBOX1* present in the promoter of the rice α-amylase (Huang et al. 1990, Lu et al. 1998, Toyofuku et al. 1998), which are responsible for inducing expression upon sugar starvation.

These different *cis*-elements were able to drive the expression of the GUS reporter gene in response to physiological concentrations of sucrose and glucose in tobacco BY-2 suspension cells (Leterrier et al. 2003). Other sugar responsive elements have been identified, as *SP8*, present in the promoters of sporamine and α-amylase genes in sweet potato (Ishiguro and Nakamura 1994), and *ACT* accessory factor, important for sucrose repression of the RUBISCO small subunit *Rcbs2* gene (Urwin and Jenkins 1997), and the coupling elements *CE*, involved in ABA control of gene expression (Niu et al. 2002).

An interesting study reported the diversity of promoter motives involved in sugar sensitivity in *Arabidopsis* (Li et al. 2006). However, these sugar responsive *cis*-regulating elements are not systematically encountered in all sugar regulated genes, and, to be functional, some require the presence of other regulatory sequences in the promoter. For instance the *AMYBOX2* sequence is often associated with the *G*- and *GC*-box motives in a sugar response sequence (*SRS*) responsive to sugar depletion in cereals (Lu et al. 1998, 2002, Toyofuku et al. 1998). Eventually, some of these elements are related to both sugar and phytohormones responses (Rook et al. 2006, Li et al. 2006). Transcription factors able to act through these sequences have been identified. They are *MYB* factors acting differentially on the *AMYBOX2* sequence in rice (Lu et al. 2002, Chen et al. 2006), WRKY proteins, able to recognize the *SURE* elements in barley (Sun et al. 2003) or bZIP proteins binding to *G*-box sequences (Lee et al. 2003). In grape, the ASR protein VvMSA induces the *VvHT1* promoter activity through the particular overlapping motifs *SURE1* and *Sucrose box 3*, and the action of a MADS-box transcription factor on the same sequence is suggested (Çakir et al. 2003, Agasse et al. 2007).

As already mentioned, VvHT1 is down-regulated by glucose at the protein level. However, whether the translational process or the turn-over is affected is still unclear. Furthermore, a concomitant effect of a putative decrease in *VvHT1* mRNA stability cannot be ruled out (Conde et al. 2006). Several examples of post-transcriptional control by sugars have been reported: (a) sugars modulate the stability of the *α-AMY3* and *Incw1* mRNAs, acting through their *3′UTR* sequences, in rice and maize respectively (Chan and Yu, 1998, Cheng et al. 1999); (b) sucrose impairs the translation of *bZIP* transcription factors through a conserved upstream *5′UTR* open reading frame (Rook et al. 1998,

Wiese et al. 2004, 2005); (c) glucose enhances the degradation of the *ETHYLENE-INSENSITIVE3* (*EIN3*) transcription factor, a component in ethylene signalling, *via* an HXK-dependent pathway (Yanagisawa et al. 2003).

Information about intermediates of sugar signalling pathways implicated in the control of sugar transport during grape maturation is scarce. Kinases, phosphorylases and calcium fluxes, widely involved as intermediate steps in signalling, may be part of sugar transport control pathways as suggested by the regulation of BvSUT1, whose activity and expression are both affected by phosphorylation (Roblin et al. 1998, Ransom-Hodgkins et al. 2003). Sugar feeding to autotrophically grown *Arabidopsis* roots induced an increase of cytosolic Ca^{2+} concentration in the whole plant (Furuichi et al. 2001).

Furthermore, a Ca^{2+}-dependent, calmodulin-independent protein kinase (CDPK) activity as well as a mitogen-activated protein kinase (MAPK)-like activity have been characterized in the developing mesocarp of grape berry (Shen et al. 2004). A Ca^{2+}-dependent protein kinase (ACPK1) whose activity is stimulated by ABA, is expressed at the plasma membrane and chloroplast/plastid membranes of berry cells in a developmental manner (Yu et al. 2006). SnRK protein kinases, identified in plants and related to the yeast SNF1 protein, a kinase involved in sugar signalling pathways, also mediate sugar signal responses (Rolland et al. 2006). Further studies, using pharmacological abolishers of phosphorylation events or Ca^{2+} fluxes for instance (okadaic acid, staurosporine, lanthane chloride) would provide important clues about their involvement in sugar-transport regulation.

Recently, the hypothesis of a favoured localization of plant sugar transporters in a specific lipidic environment, similar to the mammalian and yeast well documented lipid rafts, has been postulated. Indeed, the *Chlorella* HUP1 does not show a homogeneous distribution in the plasma membrane. A patchy distribution pattern is also observed once expressed in *S. cerevisiae* as a HUP1-GFP fusion protein and its purification from yeast membrane indicates its favoured localization with sterols and sphingolipids. Furthermore, the localization of HUP1 within the raft cluster increases the catalytic activity of the transporter in yeast (Grossmann et al. 2006). The existence of raft domains in plant plasma membranes is suggested by detergent solubility experiments (Peskan et al. 2000). In mammals and yeasts rafts may play important roles in protein trafficking, and allow protein interaction. Raft may also maintain apart proteins until a signal promotes their interaction (Opekarova and Tanner 2003). A possible involvement of raft domains in sugar signalling would deserve attention.

4.2. Sugars and production of phenolic compounds

Acquisition of the red/blue color of grape berries in red varieties along

ripening is a visual indicator of the biochemical processes occurring in grape. This reflects more precisely the accumulation of anthocyanins pigments in the vacuoles of skin cells, which does not occur in the white grape varieties. Interestingly, anthocyanins precursors and intermediates are ubiquitously synthesized within the fruit while the final pigments accumulate only in the epidermal tissue of the berries. Recently, the role of a putative anthocyanin carrier has been evidenced that would explain such phenomenon (Braidot et al. 2008). Sucrose-induced production of anthocyanins has been demonstrated in various species, such as petunia (Tsukaya et al. 1991, Weiss 2000), grape (Larronde et al. 1998, Vitrac et al. 2000), and radish (Hara et al. 2004). Expression of *CHS* is upregulated by sucrose treatment, both in petunia and *Arabidopsis* (Tsukaya et al. 1991, Ohto et al. 2001).

In *V. vinifera*, the enhanced expression of various genes of the anthocyanin biosynthetic pathway in the berry skin may be correlated with the concomitant accumulation of sugars in the flesh (Boss et al. 1996). In *V. vinifera* cell suspensions, sucrose treatment promotes anthocyanin synthesis (Larronde et al. 1998). Indeed, sucrose upregulates the expression of dihydroflavonol reductase (DFR) and anthocyanin synthase/leucoanthocyanidin dioxygenase (ANS/ LDOX). This upregulation and the accumulation of anthocyanins are sucrose specific in *Arabidopsis*. However, in grape both glucose and sucrose are able to enhance DFR and ANS expression which suggests different sugar-sensing mechanisms (Gollop et al. 2001, 2002).

In grape, the sucrose signal is transmitted *via* a pathway involving variation of Ca^{2+} concentrations and the action of kinases and phosphatases (Vitrac et al. 2000). Interestingly, in *Arabidopsis*, the sucrose transporter SUC2 is involved in anthocyanin biosynthesis: the *pho3* mutation impairs the expression of the correct SUC2 protein, resulting in an impaired phloem loading and thus a concomitant accumulation of sugars in leaves. As a result, high levels of anthocyanins are accumulated because of the specific action of sucrose on the expression of anthocyanin biosynthesis-related genes as well as on the levels of transcription factors such as *AtMYB5*, also known as *PRODUCTION OF ANTHOCYANIN 1* (*PAP1*), which is sucrose-inducible (Lloyd and Zakhleniuk 2004, Teng et al. 2005, Solfanelli et al. 2006). Furthermore, recent studies reveal the role of the transporter AtSUC1 in the sucrose-dependent signalling leading to accumulation of anthocyanins in seedlings. AtSUC1 mediates sucrose uptake into germinating pollen and its expression in roots is sucrose-inducible (Stadler et al. 1999, Johnson et al. 2004, Sivitz et al. 2008). Its activity would also trigger a sucrose-dependent transduction pathway leading to anthocyanin accumulation in cotyledons (Sivitz et al. 2008).

Interestingly, sucrose also accelerates the accumulation of carotenoids in the non-climacteric citrus fruit (Iglesias et al. 2001). Carotenoids are accumulated in chloroplasts-derived plastids, the chromoplasts, through the general iso-

prenoid biosynthetic pathway which is upregulated along fruit maturation (for review, see Cunningham and Gantt 1998). Like polyphenols, carotenoids are antioxidant molecules recommended in human nutrition and may protect against cancer and eye degenerative disease (Jonhson 2002).

Tomato fruit accumulates high levels of carotenoids (mainly lycopene) as it ripens, contributing to its characteristic red and orange color. Light and hormones are involved in the control of carotenoid biosynthesis (Giovannoni 2004); the *Never ripe* mutant (*Nr*), affected in ethylene sensitivity accumulates low levels of lycopene (Lanahan et al. 1994). Similarly, this climacteric-associated hormone also enhances anthocyanins production in grape berry (El-Kereamy et al. 2003). Sucrose promotes specifically lycopene and phytoene accumulation in tomato without affecting other carotenoids. Accordingly, sucrose enhances the accumulation of the phytoene synthase (*PSY1*) mRNAs, without acting on the expression of other carotenoid biosynthesis pathway-related genes (Télef et al. 2006).

4.3. Sugar, hormonal and environmental signalling cross-talk

Sugar signalling often inter-connects with hormone-induced pathways to modulate gene expression and physiological processes along plant life. In *Arabidopsis*, interactions between sugar and ABA signalling have been mostly studied during early seedling development through the characterization of mutants. Many of these mutations affecting sugar signalling are allelic with components of ABA synthesis or ABA transduction pathway (Leon and Sheen 2003, Rook and Bevan 2003, Gibson 2004). Glucose induces a specific accumulation of ABA during this stage of plant life, an essential increase for the plant to potentiate the efficiency of sugar signals (Rolland et al. 2006). In grape, ABA and sugars, both accumulating during berry ripening, affect important physiological processes in a coordinated manner, such as anthocyanin production and hexose transport. Indeed, in spite of some contradictory reports, it appears that sucrose and ABA act positively on polyphenols and anthocyanins accumulation (Pirie and Mullins 1976, Larronde et al. 1998, Vitrac et al. 2000, Hiratsuka et al. 2001).

A cross-talk between ABA and glucose metabolism is also suggested to enhance phenolics production (Weiss 2000). Furthermore, ABA induces the expression of *VvHT1* (Atanassova et al. 2003) and acts synergistically with sugars (sucrose and glucose at physiological concentrations) to transiently induce *VvHT1* transcription. In fact, *VvHT1* response to both signals involves the presence in its promoter of the *cis*-element *S3S1*, a specific overlapping configuration of the two sugar response *cis*-elements *Sucrose box 3* and *SURE1*. *S3S1* constitutes a target for the fixation and the action of the VvMSA (*V. vinifera*

Maturation, Stress Abscisic acid-induced, also known as VvASR, *Vitis vinifera* Abscisic acid, Stress, Ripening-induced, AF281656) protein. *VvMSA* gene expression is strongly upregulated by the combined effect of sugars and ABA and VvMSA enhances *VvHT1* promoter activity (Çakir et al. 2003).

ASRs are globular and highly hydrophilic proteins identified in many plant species but absent from *A. thaliana* and some other members of the *Brassicaceae* family (Carrari et al. 2004, Shkolnik and Bar-Zvi 2008). They are involved in abiotic stress (water deficit, salt stress) responses, senescence, pollen maturation and fruit ripening and they respond to ABA, a phytohormone involved in water deficit stresses (Iusem et al. 1993, Kalifa et al. 2004b, Yang et al. 2005), but the different homologues so far identified have different expression patterns, which are organ-specific (Maskin et al. 2001, Frankel et al. 2006). They are small enough to enter the nuclear pore and some, but not all so far identified ASRs, possess a C-terminal nuclear localization signal sequence. However, this signal is neither a sufficient nor exclusive condition for the targeting of the protein to the nucleus. Indeed, regardless the presence of a nuclear targeting signal, most ASR proteins are localized in the cytosol and the nucleus, and some are able to bind to DNA (Carrari et al. 2004). Their role as transcription regulators was first evidenced in grape (Çakir et al. 2003) and confirmed in tomato (Kalifa et al. 2004a, Rom et al. 2006).

Indeed, the tomato ASR1 (SIASR1) is a DNA binding zinc-dependent protein that preferentially binds to the $C_{2\text{-}3}$(C/G)A sequence (Kalifa et al. 2004a). The potato ci21A/Asr1 plays also a role in the control of hexose transport in heterotrophic organs, since when overexpressed, the tubers display reduced levels of plasma membrane *HT* mRNAs and consequently lower glucose uptake rates (Schneider et al. 1997, Frankel et al. 2007).

Recent data indicate that SIASR1 competes with *ABI4* transcription factor for binding the promoter *CE1 cis*-acting element, and that the overexpression of *SIASR1* in *Arabidopsis* results in an *abi4* phenotype (Shkolnik and Bar-Zvi 2008). *ABI4* transcription factors are involved in ABA sensitivity in *Arabidopsis* seedlings and many *abi4* allelic mutations were discovered while screening for mutants impaired in sugar and salt signalling, suggesting that the ABI4 protein is a cross-road between these pathways (Finkelstein et al. 1998, Quesada et al. 2000, Niu et al. 2002, Barrero et al. 2006). Indeed, a common feature for all ASR proteins is their response to ABA.

In grape suspension cultured cells, *VvASR* expression is strongly enhanced by the addition of ABA. In ripening berries, ABA and sugars accumulate simultaneously after véraison and it is tempting to associate the high expression of *VvASR* at this time with the presence of both compounds. One should keep in mind however, that the expression patterns displayed by *VvASR* and *VvHT1* in grape berry along ripening are strictly opposite to each other, which, together with the role of VvASR on *VvHT1*'s transcription, can consti-

tute a brain-teasing problem. Interestingly, the potato ASR *ci21A/Asr1* (orthologue of tomato *Asr1*) expression is also negatively correlated with *HT* mRNAs accumulated along fruit maturation (Frankel et al. 2007).

However, it is important to recall that positive effect of VvASR on *VvHT1* transcription was demonstrated in tobacco, a heterologous system where the protein may not have been in contact with its natural partners. Furthermore, in the light of recent results (Shkolnik and Bar-Zvi 2008), it is possible that *VvASR*, as *SlASR1*, is able to compete with other transcription factors for preferred promoter binding sites. Eventually, additional roles, such as a modulation of DNA topology at the image of non-histone chromosomal proteins in response to water and salt stresses, as well as a protective role against water loss cannot be ruled out. ASRs are members of hydrophilins proteins, and enhance water retention once over-expressed, due to their highly hydrophilic nature (Gilad et al. 1997, Koag et al. 2003, Carrari et al. 2004, Kalifa et al. 2004b, Yang et al. 2005, Frankel et al. 2007, Maskin et al. 2007).

Additional connections with the sugar signalling pathways are demonstrated mostly in *Arabidopsis* with hormones, such as auxins and gibberellins (GAs), cytokinins, ethylene and brassinosteroids. The connection occurs either through the allelic mutation of a gene or the involvement of common *cis*-acting elements: the *AMYBOX2* (*TATCCA*) sequence is also involved in GAs sensitivity of the *α-Amy3* gene encoding for an amylase in rice (Gubler and Jacobsen 1992). The *DRE*-related element (Drought Response Element) conferred glucose-, ABA- and water stress response (Seki et al. 2007). Recent data reported by Li and co-workers (2006) propose a detailed study of *cis*-acting elements that confer gene response to glucose and ABA or light signalling as well as transcription factors involved in *A. thaliana.* Indeed, their microarray studies demonstrate the close connection between these pathways at the levels of promoter, *trans*-acting elements and gene expression patterns.

In *A. thaliana*, the *hls1* mutant is affected in both sugar and auxin signalling (Ohto et al. 2006). In grape berry and tomato fruit, an early treatment with auxin delays the accumulation of both sugars and secondary metabolites (Cohen 1996, Davies et al. 1997). Besides its positive effect on anthocyanin production, ethylene induces the expression of the sucrose transporters *VvSUC11* and *VvSUC12* and sugar accumulation in grape (Chervin et al. 2006). Furthermore, this climacteric-associated hormone upregulates the alcohol dehydrogenase gene, allowing the further development of aroma and participates in the control of berry acidity (Chervin et al. 2004, Tesnière et al. 2004). Some *A. thaliana* mutants affected in the perception (Zhou et al. 1998) or the transduction of ethylene, are hypersensitive to sugars, pointing to the existence of negative interactions between both signal transduction pathways (Gibson et al. 2001, Leclercq et al. 2002, Yanagisawa et al. 2003).

In tomato, the *ripening-inhibitor* (*rin*) mutation impairs the typical ripening-associated increase in ethylene production that occurs during climacteric fruit maturation and the fruit does not ripen. The mutation affects a gene encoding for a MADS-box transcription factor. Such proteins are widespread in all eukaryotic kingdoms and involved in growth and developmental processes. In plants, they were first characterized in the context of floral initiation and meristem differentiation, and recently implicated in developmental, non-hormonal control of fruit ripening (Vrebalov et al. 2002, Giovannoni 2004). In grape, although many genes encoding MADS-box proteins are expressed in flower, some are found in the fruit: *VvMADS5* is expressed during early berry development while *VvMADS1* and *VvMADS4* expression is strong throughout development (Boss et al. 2001, 2002, 2003, Chatelet et al. 2007). However, there is no information available about its putative involvement in ethylene signalling.

Processes like the accumulation of sugars, the metabolism of organic acids, the synthesis of aroma compounds and the accumulation of anthoyanins in the skin of grape appear to be controlled by brassinosteroids (BRs; Symons et al. 2006). The role of these hormones in both climacteric and non-climacteric fruits ripening processes is also evidenced. Indeed, application of these compounds promotes tomato fruit and grape berry ripening, increases sugar levels and decreases acid contents in both species. They also enhance the accumulation of lycopene in the pericarp of tomato fruit and of anthocyanins in grape berry (McMorris 1997, Vidya Vardhini and Rao 2002, Symons et al. 2006, Pilati et al. 2007). In tomato however, their effect on fruit ripening could be indirect, through the increase of ethylene levels (Vidya Vardhini and Rao 2002). In grape, there is a dramatic increase of some BRs at the onset of ripening (Symons et al. 2006).

The characterization of pleiotropic mutations also provides clues regarding the complexity of inter-connections of sugars, hormonal and environmental transduction pathways. Besides being involved in sugar signalling, the *prl1* (Németh et al. 1998), *gin2* (Moore et al. 2003), *ctr1* (Gibson et al. 2001, Leclercq et al. 2002) and *bls1* (Laxmi et al. 2004) mutations are also implicated in hormonal and environmental responses. The discovery that a SNF1-Related kinase complex is also differentially regulated by ABA and GAs provides strong evidence for a link between hormonal and sugar-sensing pathways controlling seed development, dormancy, and germination in tomato (Bradford et al. 2003).

5. PERSPECTIVES

The characterization of mutants provided considerable information allowing the understanding of major developmental and physiological processes by

functional genomics in *A. thaliana*. Molecular and genetic understanding of fleshy fruits maturation also made significant progress thanks to tomato mutants affected in fruit set and maturation. However, information regarding non-climacteric fruits is still scarce.

The characterization of natural and artificial mutants affected in berry development and/or ripening would provide an important tool for studying the molecular processes involved. For instance, the natural mutant Pinot Meunier *Vvgai* results in a dwarf and a short-cycling phenotype. Due to its short-cycling and its reduced size, this mutant, which is not affected in berry growth, is a promising tool to accelerate transgenic approaches on genes controlling berry development (Boss and Thomas 2002).

The grape mutant *fleshless berry* (*flb*) is affected in many aspects and constitutes an interesting model. Indeed, this loss-of-function mutation alters flesh development without impairing the production of normal number and fully developed seeds. The final volume of these berries is reduced to 10 % of control values, whereas softening of the pseudo-flesh and colour change are not affected. Malate accumulation in the mutant is reduced at green stage compared to the wild-type; sucrose contribution to the total sugar of the berry is increased in the ripe mutant whereas hexoses are mostly accumulated in the wild-type (Fernandez et al. 2006). The mutation promotes the expression of several genes related to ripening and/or to stress and impairs the expression of several regulatory genes. The *fleshless berry* mutant appears then a good model to identify genes putatively involved in the early development of grapevine fruit (Fernandez et al. 2007).

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